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(54) 【発明の名称】 抗原又は抗体の検出シート、検出キット及び検出方法

(57) 【要約】

【課題】 簡単な操作で高感度で生物学的液体中の抗原又は抗体を検出する検出シート、検出キット及び検出方法を提供すること。

【解決手段】 平均粒径が0.01 $\mu$ m~200 $\mu$ mでCa/P比が1.0~2.0のリン酸カルシウム系化合物粒子を担持した繊維集合体にアビジン又はストレプトアビジンが固定されていることを特徴とする抗原又は抗体の検出シートである。固定されたアビジン又はストレプトアビジンに、ビオチン化された抗原又は抗体を結合させた後、検出シートを試料溶液と接触させることにより試料中の抗体又は抗原を結合させ、その後、該抗体又は抗原と特異的に結合する標識化合物の溶液と接触させ、標識された抗原-抗体複合体を検出する。

## 【特許請求の範囲】

【請求項1】 平均粒径が $0.01\mu\text{m}\sim 200\mu\text{m}$ でCa/P比が $1.0\sim 2.0$ のリン酸カルシウム系化合物粒子を担持した繊維集合体にアビジン若しくはストレプトアビジン又はこれらの誘導体が固定されていることを特徴とする抗原又は抗体の検出シート。

【請求項2】 固定されたアビジン若しくはストレプトアビジン又はこれらの誘導体に、ビオチン化された抗原又は抗体を結合させた請求項1記載の抗原又は抗体の検出シート。

【請求項3】 繊維集合体の裏面に補強用フィルム又はシートを有する請求項1又は2記載の抗原又は抗体の検出シート。

【請求項4】 請求項2又は3記載の抗原又は抗体の検出シートと標識化合物溶液とからなる抗原又は抗体の検出キット。

【請求項5】 請求項2又は3記載の抗原又は抗体の検出シートを試料溶液と接触させることにより試料中の抗体又は抗原を結合させ、その後、該抗体又は抗原と特異的に結合する標識化合物の溶液と接触させ、標識された抗原-抗体複合体を検出することを特徴とする抗原又は抗体の検出方法。

## 【発明の詳細な説明】

## 【0001】

【発明の属する技術分野】本発明は、唾液、血液、リンパ液、糞尿などの生物学的液体中の抗原又は抗体を検出する検出シート、検出キット及び検出方法に関する。

## 【0002】

【従来の技術】近年、抗原抗体反応を利用した様々な臨床検査が行われているが、特別の施設や臨床検査技師が必要である。そのため、特別の施設がなく、臨床検査技師がいない小病院でも容易に簡単な操作で検査を行うことができ、病気診断の一助にしようとする高感度な検査シートが求められている。ハイドロキシアパタイト等のリン酸カルシウム系化合物が蛋白質、核酸などに対して優れた吸着能を有することから、本発明者らは紙や不織布に担持させたリン酸カルシウム系化合物粒子に抗原又は抗体を吸着させ、抗原抗体反応を行わせる検出シートを既に提案している（特願平6-214706号明細書参照）しかしながら、リン酸カルシウム系化合物は酸性蛋白質に対しては吸着能が不十分であり、また、抗体の吸着に際して吸着部位が必ずしも一定せず、例えば、Fabフラグメント部分を吸着することがあるなど、配向性に問題があることが分かった。したがって、リン酸カルシウム系化合物自体では吸着されにくい抗原又は抗体の固定法あるいはリン酸カルシウム系化合物と抗体の有効な結合方法になお改善の余地が残されていた。

## 【0003】

【発明が解決しようとする課題】本発明は、上記の従来技術の問題点を解消し、簡単な操作で高感度で生物学的

液体中の抗原又は抗体を検出しようとする検出シート、検出キット及び検出方法を提供することを目的とする。

## 【0004】

【課題を解決するための手段】本発明は、繊維集合体に担持したリン酸カルシウム系化合物にアビジン又はストレプトアビジンを吸着固定しておくことによって上記目的を達成しようとするの知見に基づいて完成したものである。すなわち、本発明による抗原又は抗体の検出シートは、平均粒径が $0.01\mu\text{m}\sim 200\mu\text{m}$ でCa/P比が $1.0\sim 2.0$ のリン酸カルシウム系化合物粒子を担持した繊維集合体にアビジン若しくはストレプトアビジン又はこれらの誘導体が固定されていることを特徴とする。

【0005】本発明の検出シートは、上記のように固定されたアビジン若しくはストレプトアビジン又はこれらの誘導体に、ビオチン化された抗原又は抗体を結合させた状態で提供することもできる。また、本発明による抗原又は抗体の検出方法は、本発明による抗原又は抗体の検出シートを試料溶液と接触させることにより試料中の抗体又は抗原を結合させ、その後、該抗体又は抗原と特異的に結合する標識化合物の溶液と接触させ、標識された抗原-抗体複合体を検出することを特徴とする。

## 【0006】

【発明の実施の形態】本発明においては、リン酸カルシウム系化合物にアビジン若しくはストレプトアビジン又はこれらの誘導体を吸着させる。ここで、誘導体としては、アビジンより糖鎖部分を除いたもの、例えばニュートラアビジン（NeutrAvidin）、ウルトラアビジンなどが挙げられる。

【0007】本発明においては、アビジン若しくはストレプトアビジン又はこれらの誘導体の吸着固定剤としてリン酸カルシウム系化合物粒子を使用する。ここで、リン酸カルシウム系化合物としては、Ca/P比が $1.0\sim 2.0$ であれば各種のリン酸カルシウム系化合物を使用することができ、例えば、 $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ 、 $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$ 、 $\text{Ca}_{10}(\text{PO}_4)_6\text{Cl}_2$ 、 $\text{Ca}_3(\text{PO}_4)_2$ 、 $\text{Ca}_2\text{P}_2\text{O}_7$ 、 $\text{Ca}_4\text{O}(\text{PO}_4)_2$  及び  $\text{CaHPO}_4$  のうちから選ばれた1種又は2種以上を使用することができる。これらのうちハイドロキシアパタイト及びリン酸三カルシウムが好ましく、特にハイドロキシアパタイトを主成分とするものが最も好ましい。フッ素アパタイトを用いる場合、全リン酸カルシウム系化合物中のフッ素含有率が5重量%以下であるのが好ましい。フッ素含有率が5重量%を超えると、フッ素の溶出が起り好ましくない。これらのリン酸カルシウム系化合物は、公知の湿式合成法、乾式合成法などによって合成することができる。

【0008】リン酸カルシウム系化合物の粒子は、例えばリン酸カルシウム系化合物のスラリーを噴霧乾燥することによって造粒し、これを焼成することによって調製

することができるが、この方法に限らず他の造粒法によって調製することも可能である。なお、ふるい分けなどの手段により、粒子の粒度を目的に応じて所定の範囲に選定して用いることがより好ましい。使用するリン酸カルシウム系化合物粒子は、平均粒径が $0.01 \sim 200 \mu\text{m}$ であるのが好ましい。平均粒径が $0.01 \mu\text{m}$ 未満であると、粒子が凝集しやすくなり均一に担持されなくなり、担持率が著しく低下する。

【0009】さらに、リン酸カルシウム系化合物粒子は、リン酸カルシウム系化合物粒子が比表面積 $10 \text{m}^2/\text{g}$ 以上で、細孔径 $500 \sim 1000 \text{\AA}$ の、一次粒子が凝集結合した多孔質粒子であるのが好ましい。比表面積が $10 \text{m}^2/\text{g}$ 未満では十分な吸着能が望めない。また、蛋白質などが吸着されて気孔内へ入り込むためには、細孔径 $500 \sim 1000 \text{\AA}$ 程度の気孔を有するのが好ましい。このような多孔質粒子は、任意の公知方法で製造することができる。

【0010】本発明においては、上記のようなリン酸カルシウム系化合物粒子の担体として繊維集合体を用いる。ここで、使用しうる繊維集合体としては、紙又は不織布が挙げられる。紙にリン酸カルシウム系化合物粒子を担持させる方法としては、リン酸カルシウム系化合物粒子を填料として用い、これを内添方式又は塗工方式により添加して紙を製造する方法がある。内添方式の場合には、リン酸カルシウム系化合物粒子及び他の添加剤を添加し、充分混合した後、通常の抄紙機を用いて製造することができる。また、塗工方式を採用する場合には、結合剤と組み合わせて原紙上に塗布すればよい。結合剤としては、特に制限はなく、例えば、ポリアクリル酸ナトリウム、ポリビニルアルコール、ラテックス、ポリアクリル酸、ポリエチレンオキシド、カルボキシメチルセルロース、ポリエステルなどを使用することができる。

【0011】不織布にリン酸カルシウム系化合物粒子を担持させる場合にも、紙の場合と同様の方法を採用することができるが、さらに、原料繊維の少なくとも1部分が熱可塑性高分子繊維から成る不織布の少なくとも表面上にリン酸カルシウム系化合物粒子を乾式法又は湿式法で担持させ、次いで、熱処理により不織布中の熱可塑性高分子繊維の少なくとも表面を軟化させて該繊維表面にリン酸カルシウム系化合物粒子を固着させる方法を採用するのが好ましい。

【0012】繊維集合体へのリン酸カルシウム系化合物の担持量は、通常、 $1 \sim 65$ 重量%、好ましくは $5 \sim 50$ 重量%とするのが好ましい。リン酸カルシウム系化合物の担持量が $1$ 重量%未満では、アビジン若しくはストレプトアビジン又はこれらの誘導体を吸着しにくくなり、また、 $65$ 重量%を超えるとアビジン若しくはストレプトアビジン又はこれらの誘導体の使用量が高くなるため経済的ではない。

【0013】こうして製造した繊維集合体の少なくとも表面には、上記のように、リン酸カルシウム系化合物層を有し、リン酸カルシウム系化合物は蛋白質の一種であるアビジン、ストレプトアビジン及びこれらの誘導体に対して高い吸着作用を有し、これらを吸着固定することができる。リン酸カルシウム系化合物を担持した繊維集合体にアビジン若しくはストレプトアビジン又はこれらの誘導体を固定するには、アビジン若しくはストレプトアビジン又はこれらの誘導体の水溶液を用いて浸漬、刷毛塗り、スプレー塗布などの方法で行うことができる。また、アビジン若しくはストレプトアビジン又はこれらの誘導体の固定量は、繊維集合体に担持されているリン酸カルシウム系化合物の種類及び量によって変動し、例えば、ハイドロキシアパタイト $3.0 \text{g}/\text{m}^2$  担持不織布においては、 $5 \sim 20 \text{mg}/\text{m}^2$  が好ましく、 $10 \text{mg}/\text{m}^2$  前後がより好ましい。 $5 \text{mg}/\text{m}^2$  未満では、ハイドロキシアパタイトの未吸着部位が多く、 $20 \text{mg}/\text{m}^2$  を超えると、アビジン若しくはストレプトアビジン又はこれらの誘導体量は不経済である。

【0014】アビジン、ストレプトアビジン及びこれらの誘導体は、ビオチンに対して極めて高い親和性を有し、他方、ビオチンは抗体や多くの酵素に容易に結合させることができるので、上記のように固定されたアビジン若しくはストレプトアビジン又はこれらの誘導体と、ビオチン化した抗原又は抗体とを反応させることにより、繊維集合体上に正しい配向性を以て固定化された抗原又は抗体を有する検査シートが得られ、これを試料溶液と接触させた後、標識化合物溶液と接触させることにより、容易に抗体又は抗原を高感度で検出することができる。

【0015】本発明においては、必要に応じて、ビオチン化した抗原又は抗体と反応させる前又は反応させた後に、繊維集合体に担持したリン酸カルシウム系化合物粒子の未吸着部位をブロッキング剤でマスキングすることもできるが、この工程は必ずしも必要ではない。また、本発明の検出シートの取扱性の向上のため、検出シートの不織布の裏面に補強用フィルム又はシートを付着させることもできる。補強用フィルム又はシートは、紙又はプラスチックから成るものであってよい。

【0016】したがって、本発明により、上記のような抗原又は抗体の検出シートと標識化合物溶液とからなる抗原又は抗体の検出キットを提供することができる。標識化合物としては、試料中の抗原又は抗体と特異的に結合する酵素標識抗体又は抗原、同位元素標識抗体又は抗原などを使用することができるが、特別の設備を必要とすることなく、簡単に検出操作を行ないうることから酵素標識が好ましい。酵素標識抗体若しくは抗原を用い、その酵素の可視光又は紫外領域において呈色する基質を用いて目視観察あるいは吸光度測定などの簡単な方法で検出することができる。



【0017】標識化合物として用いられる酵素と基質としては、例えば、酵素アルカリフォスファターゼに対しては基質BCIP及びNBT、又はDNPが用いられ、酵素西洋わさびペルオキシダーゼに対しては基質OPD、DAB又は4CNが用いられ、 $\beta$ -ガラクトシダーゼに対しては基質pNPG、X-gal又はBluo-galが用いられる。

【0018】本発明による検出シート及び検査方法は、主として抗原又は抗体の存否を検出するものであるが、呈色の濃度の比較や吸光度の測定によって抗原又は抗体のある程度の定量も可能である。

#### 【0019】

【実施例】次に、実施例に基づいて本発明をさらに詳述するが、本発明はこれによって制限されるものではない。

#### 【0020】実施例1

ポリエチレン50重量%とポリエチレンテレフタレート50重量%から成る厚さ0.2mmの不織布にほぼ一様に平均粒径 $3.5\mu\text{m}$ 、Ca/P比1.67の多孔質ハイドロキシアパタイト顆粒を加熱処理により24重量%担持させたアパタイト担持不織布（大きさ $5\text{mm}\times 5\text{mm}$ ）をアビジン $5\mu\text{g}/\text{ml}$ の水溶液中に浸漬してアビジン $10\text{mg}/\text{m}^2$ が吸着された不織布を作成し、これにウサギのビオチン化抗ロタ（Rota）IgGの充分量を結合させ、ロタウイルス検出用検査シートを作成した。得られた検査シートを用いてサンドイッチEIA法を用いてロタウイルスを検出した。すなわち、ロタウイルス溶液中のロタウイルス抗原を上記検出シートで捕捉する。その後モルモットの抗ロタウイルス・アルカリフォスファターゼ標識抗体を検出シート上のロタウイルス抗原と結合させたのち、EIA用基質DNPの発色により測定した。測定波長は $405\text{nm}$ であった。ロタウイルス抗原の希釈度を変え、検出シートの感度を検討したと\*

\*ころ、 $0.062\mu\text{g}/\text{ml}$ のウイルス量まで感知可能であった。上記の測定によるウイルス濃度と吸光度との関係図として図1に示す。

#### 【0021】比較例1

ポリエチレン50重量%とポリエチレンテレフタレート50重量%から成る厚さ0.2mmの不織布にほぼ一様に平均粒径 $3.5\mu\text{m}$ 、Ca/P比1.67の多孔質ハイドロキシアパタイト顆粒を加熱処理により24重量%担持させたアパタイト担持不織布（大きさ $5\text{mm}\times 5\text{mm}$ ）にウサギの抗ロタ（Rota）IgG抗体の充分量を吸着させた後、グルタルアルデヒド0.05%溶液に浸漬処理を行い、上記繊維集合体の抗ロタ抗体未吸着部位をマスキングするためにブロックエース4倍希釈に浸漬して、ロタウイルス検出シートを作成した。得られたロタウイルス検出シートを用いて、実施例1と同様の検出条件で、ロタウイルス $0.25\mu\text{g}/\text{ml}$ の検出を試みたが、吸光度は0.01であり、検出できなかった。一方、実施例1によって作成された検出シートを用いてロタウイルス $0.25\mu\text{g}/\text{ml}$ の検出を行なったところ、吸光度は0.95であり、充分に検出を行なうことができた。

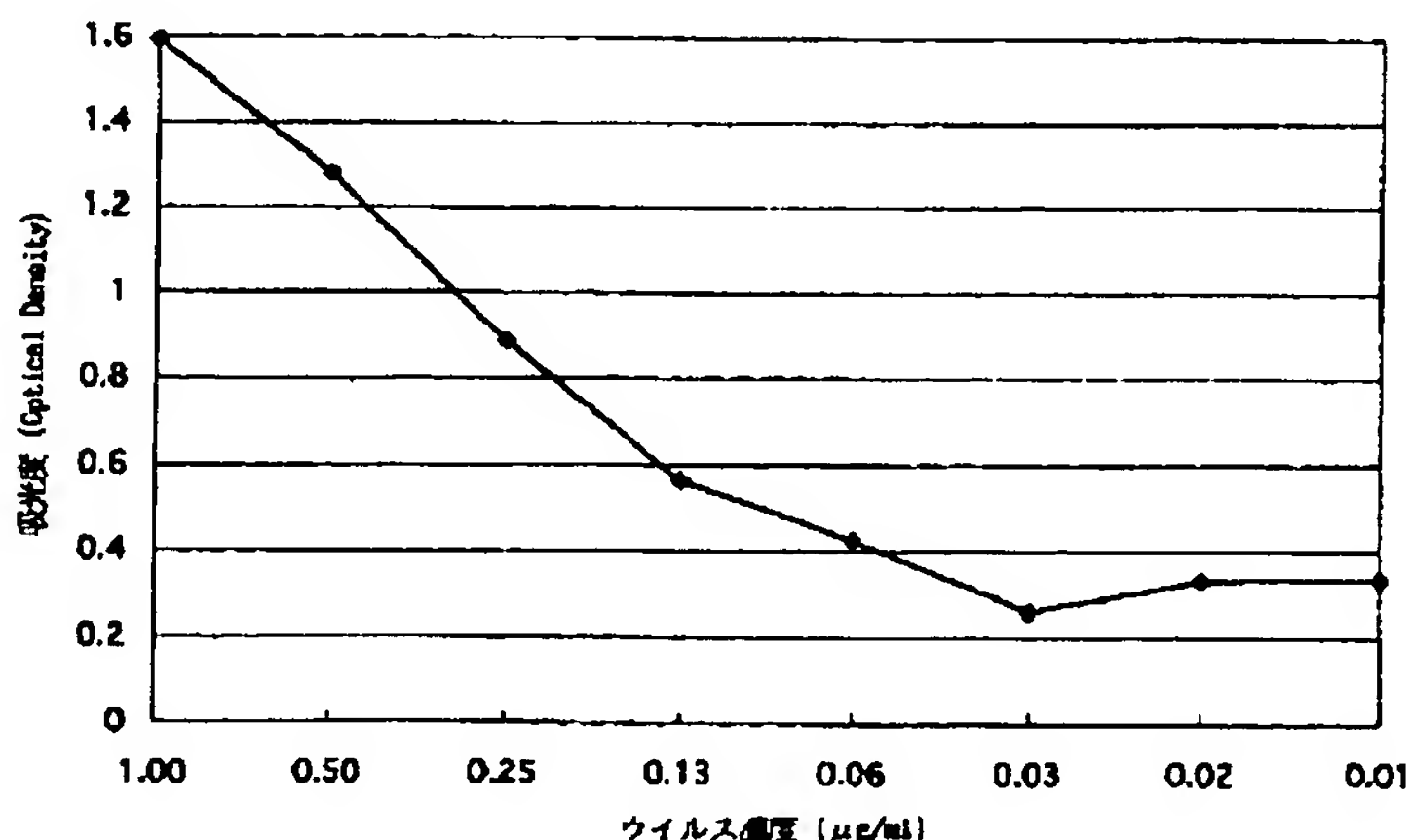
#### 【0022】

【発明の効果】本発明の検出シートを用いれば、固定化すべき抗原や抗体の種類に制限がなく、また、配向性の問題もなく、簡単な操作で、迅速に高感度で生物学的液体中の抗原又は抗体を検出することができ、検査技師などの熟練者のいない小規模病院でも抗原又は抗体の検出を容易に行ないうる安価で高感度な検出キットを提供することができる。

#### 【図面の簡単な説明】

【図1】実施例1におけるロタウイルスの検出結果を示すグラフ図である。

【図1】



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# PATENT ABSTRACTS OF JAPAN

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## (54) SHEET, KIT, AND METHOD FOR DETECTING ANTIGEN OR ANTIBODY

### (57)Abstract:

**PROBLEM TO BE SOLVED:** To provide a sheet, kit, and method for detecting antigen or antibody by which an antigen or antibody contained in a biological solution can be detected with high sensitivity through simple operation.

**SOLUTION:** A sheet for detecting antigen or antibody is constituted by immobilizing avidin or streptavidin to a fiber aggregate carrying particles of a calcium phosphate compound having an average particle diameter of 0.01-200 $\mu$ m and a Ca/P ratio of 1.0-2.0. By bonding an antibody or antigen contained in a sample solution to the immobilized avidin or streptoavidin by bringing the sheet into contact with the sample solution after bonding a biotinized antigen or antibody to the avidin or streptoavidin and bringing the sheet into contact with the solution of a labeled compound which is specifically bonded to the antibody or antigen in the sample solution, a labeled antigen- antibody complex is detected.

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**CLAIMS**

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[Claim(s)]

[Claim 1] The detection sheet of the antigen or antibody characterized by fixing avidin, streptoavidin, or these derivatives to the fiber aggregate with which the calcium/P ratio supported [ mean particle diameter ] the calcium phosphate system compound particle of 1.0–2.0 with 0.01 micrometers – 200 micrometers.

[Claim 2] The antigen according to claim 1 which combined the antigen or antibody biotin-ized with the fixed avidin, streptoavidin, or these derivatives, or the detection sheet of an antibody.

[Claim 3] The antigen according to claim 1 or 2 which has a film for reinforcement, or a sheet at the rear face of the fiber aggregate, or the detection sheet of an antibody.

[Claim 4] The detection kit of the antigen or antibody which consists of an antigen according to claim 2 or 3, or the detection sheet and labeled compound solution of an antibody.

[Claim 5] The detection approach of the antigen or antibody characterized by detecting the antigen-antibody complex by which was made to combine the antibody or antigen in a sample, and was contacted in the solution of the labeled compound specifically combined with this antibody or an antigen after that, and the indicator was carried out by contacting an antigen according to claim 2 or 3 or the detection sheet of an antibody to the sample solution.

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**DETAILED DESCRIPTION**

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[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention relates to the detection sheet, detection kit, and the detection approach of detecting the antigen or antibody in biological liquids, such as saliva, blood, lymph, and feces and urine.

[0002]

[Description of the Prior Art] Although various clinical laboratory tests using an antigen-antibody reaction are conducted in recent years, a special facility and a special clinical laboratory technologist are required. Therefore, there is no special facility, it can inspect by easy actuation easily also in the small hospital in which a clinical laboratory technologist is not present, and the high sensitivity inspection sheet which can be made into an aid of a sick diagnosis is called for. Since it has the adsorption capacity in which calcium phosphate system compounds, such as hydroxyapatite, were excellent to protein, a nucleic acid, etc., this invention persons made the antigen or the antibody stick to the calcium phosphate system compound particle which paper and a nonwoven fabric were made to support, and the detection sheet on which an antigen-antibody reaction is made to perform has already been proposed (refer to Japanese-Patent-Application-No. No. 214706 [ six to ] specification).

However, it turned out that a problem is in that a calcium phosphate system compound has inadequate adsorption capacity, its adsorption site may not necessarily be fixed on the occasion of adsorption of an antibody to acidic protein, for example, a Fab fragment part may be adsorbed, and a stacking tendency. Therefore, in addition, the room of an improvement was left behind to the fixing method of the antigen which is hard to adsorb, or an antibody, or the effective joint approach of a calcium phosphate system compound and an antibody with the calcium phosphate system compound itself.

[0003]

[Problem(s) to be Solved by the Invention] This invention cancels the trouble of the above-mentioned conventional technique, and aims at offering the detection sheet, detection kit, and the detection approach high sensitivity can detect the antigen or antibody in a biological liquid by easy actuation.

[0004]

[Means for Solving the Problem] This invention is completed based on knowledge that the above-mentioned purpose can be attained by carrying out adsorption immobilization of avidin or the streptoavidin to the calcium phosphate system compound supported to the fiber aggregate. That is, the antigen by this invention or the detection sheet of an antibody is characterized by fixing avidin, streptoavidin, or these derivatives to the fiber aggregate with which the calcium/P ratio supported [ mean particle diameter ] the calcium phosphate system compound particle of 1.0-2.0 with 0.01 micrometers - 200 micrometers.

[0005] The detection sheet of this invention can also be offered where the antigen or antibody biotin-ized is combined with the avidin fixed as mentioned above, streptoavidin, or these derivatives. Moreover, by contacting the antigen by this invention, or the detection sheet of an antibody to the sample solution, the detection approach of the antigen by this invention or an



antibody combines the antibody or antigen in a sample, is contacted in the solution of the labeled compound specifically combined with this antibody or an antigen after that, and is characterized by detecting the antigen-antibody complex by which the indicator was carried out.

[0006]

[Embodiment of the Invention] Avidin, streptoavidin, or these derivatives are made to stick to a calcium phosphate system compound in this invention. Here, as a derivative, the thing (NeutrAvidin), for example, new trad avidin, excluding the sugar chain part from avidin, ultra avidin, etc. are mentioned.

[0007] In this invention, a calcium phosphate system compound particle is used as avidin, streptoavidin, or an adsorption fixative of these derivatives. Here as a calcium phosphate system compound If calcium/P ratios are 1.0–2.0, various kinds of calcium phosphate system compounds can be used. For example, calcium<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub> and calcium<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>F<sub>2</sub>, calcium<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>Cl<sub>2</sub>, calcium<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>, calcium two P<sub>2</sub>O<sub>7</sub>, and calcium 4O(PO<sub>4</sub>)<sub>2</sub> And CaHPO<sub>4</sub> One sort chosen from inside or two sorts or more can be used. Hydroxyapatite and tricalcium phosphate are [ among these ] desirable, and what uses especially hydroxyapatite as a principal component is the most desirable. When using a fluorine apatite, it is desirable that the fluorine content in a total phosphorus acid calcium system compound is 5 or less % of the weight. The elution of a fluorine happens and is not desirable if fluorine content exceeds 5 % of the weight. These calcium phosphate system compounds are compoundable with a well-known wet synthesis method, a dry type synthesis method, etc.

[0008] Although it can prepare by corning the particle of a calcium phosphate system compound by carrying out spray drying of the slurry of for example, a calcium phosphate system compound, and calcinating this, not only this approach but the thing to prepare by other corning methods is possible. In addition, it is more desirable to select and use for the predetermined range [ for the purpose of the grain size of a particle ] with means, such as sieving. As for the calcium phosphate system compound particle to be used, it is desirable that mean particle diameter is 0.01–200 micrometers. It becomes it easy to condense a particle that mean particle diameter is less than 0.01 micrometers, and is not supported by homogeneity. Moreover, if it exceeds 200 micrometers, it will be hard coming to support to a nonwoven fabric, and the rate of support will fall remarkably.

[0009] Furthermore, calcium phosphate system compound particles are more than specific surface area of 10m<sup>2</sup> / g, and, as for a calcium phosphate system compound particle, it is desirable that it is the porosity particle in which the primary particle with a pole diameter of 500–1000Å carried out condensation association. Adsorption capacity with a specific surface area sufficient by under 10m<sup>2</sup> / g cannot be desired. Moreover, in order to adsorb and for protein etc. to enter into pore, it is desirable to have pore with a pole diameter of about 500–1000Å. Such a porosity particle can be manufactured by the well-known approach of arbitration.

[0010] In this invention, the fiber aggregate is used as support of the above calcium phosphate system compound particles. Here, paper or a nonwoven fabric is mentioned as the fiber aggregate which can be used. There is a method of adding this with an inner attachment method or a coating method as an approach of making paper supporting a calcium phosphate system compound particle, using a calcium phosphate system compound particle as a loading material, and manufacturing paper. In the case of an inner attachment method, after adding a calcium phosphate system compound particle and other additives and mixing enough, it can manufacture using the usual paper machine. Moreover, what is necessary is just to apply in the Hara paper combining a binder, in adopting a coating method. As a binder, there is especially no limit, for example, it can use sodium polyacrylate, polyvinyl alcohol, a latex, polyacrylic acid, polyethylene oxide, a carboxymethyl cellulose, polyester, etc.

[0011] Although the same approach as the case of paper can be adopted also when making a nonwoven fabric support a calcium phosphate system compound particle Even if there are few nonwoven fabrics with which at least 1 part of raw material fiber consists of thermoplastic macromolecule fiber, a calcium phosphate system compound particle is made to support with dry process or a wet method on a front face. Furthermore, subsequently It is desirable to adopt the approach of the thermoplastic macromolecule fiber in a nonwoven fabric of softening a front face

at least and making this fiber front face fixing a calcium phosphate system compound particle, by heat treatment.

[0012] As for the amount of support of the calcium phosphate system compound to the fiber aggregate, it is usually preferably desirable to consider as 5 – 50 % of the weight one to 65% of the weight. At less than 1 % of the weight, since the amount of avidin, streptoavidin, or these derivatives used will become high if it is hard coming to adsorb avidin, streptoavidin, or these derivatives and exceeds 65 % of the weight, the amount of support of a calcium phosphate system compound is not economical.

[0013] In this way, it has a high absorption to the avidin of the manufactured fiber aggregate whose calcium phosphate system compound it has a calcium phosphate system compound layer as mentioned above in a front face, and is a proteinic kind at least, streptoavidin, and these derivatives, and adsorption immobilization of these can be carried out. In order to fix avidin, streptoavidin, or these derivatives to the fiber aggregate which supported the calcium phosphate system compound, it can carry out by the approach of immersion, brush coating, a spray coating cloth, etc. using the water solution of avidin, streptoavidin, or these derivatives. Moreover, avidin, streptoavidin, or the amount of immobilization of these derivatives is changed with the class and amount of a calcium phosphate system compound which are supported by the fiber aggregate, for example, is hydroxyapatite 3.0 g/m<sup>2</sup>. It sets to a support nonwoven fabric and is 5 – 20 mg/m<sup>2</sup>. It is desirable and is 10 mg/m<sup>2</sup>. Order is more desirable. 5 mg/m<sup>2</sup> There are many non-adsorption sites of hydroxyapatite at the following, and it is 20 mg/m<sup>2</sup>. If it exceeds, avidin, streptoavidin, or these amounts of derivatives are uneconomical.

[0014] Avidin, streptoavidin, and these derivatives Since it has very high compatibility to a biotin and another side and a biotin can be easily combined with the enzyme of an antibody or many By making the avidin fixed as mentioned above, streptoavidin or these derivatives, and the antigen or antibody biotin-ized react a fiber aggregate top -- a right stacking tendency -- with, after obtaining the inspection sheet which has the fixed antigen or antibody and contacting this to the sample solution, an antibody or an antigen is easily detectable by high sensitivity by making a labeled compound solution contact.

[0015] In this invention, although the antigen or antibody biotin-ized if needed, and the front stirrup made to react can also mask the non-adsorption site of the calcium phosphate system compound particle supported to the fiber aggregate by the blocking agent after making it react, this process is not necessarily required. Moreover, the film for reinforcement or a sheet can also be made to adhere to the rear face of the nonwoven fabric of a detection sheet for improvement in the handling nature of the detection sheet of this invention. The film for reinforcement or a sheet may consist of paper or plastics.

[0016] Therefore, the detection kit of the antigen or antibody which consists of the above antigens, or the detection sheet and labeled compound solution of an antibody can be offered by this invention. Although the enzyme labelled antibody specifically combined with the antigen in a sample or an antibody as a labeled compound or an antigen, an isotope labelled antibody, or an antigen can be used, since detection actuation can be performed easily, enzyme labeling is desirable, without needing a special facility. It is detectable by easy approaches, such as visual observation or spectrometry, using the substrate which carries out coloration in the light or the ultraviolet region of the enzyme using an enzyme labelled antibody or an antigen.

[0017] As the enzyme used as a labeled compound, and a substrate, Substrates BCIP, NBT, or DNP are used to enzyme alkaline phosphatase, Substrates OPD and DAB or 4CN(s) are used to enzyme horseradish peroxidase, and Substrate pNPG, X-gal, or Bluo-gal is used to the beta-galactosidase, for example.

[0018] Although the detection sheet and the inspection approach by this invention mainly detect the existence or nonexistence of an antigen or an antibody, a certain amount of quantum of an antigen or an antibody is also possible for them by the comparison of the concentration of coloration, or measurement of an absorbance.

[0019]

[Example] Next, this invention is not restricted by this although this invention is further explained in full detail based on an example.



[0020] At about 1 appearance to a nonwoven fabric with a thickness of 0.2mm which consists of 50 % of the weight of example 1 polyethylene, and 50 % of the weight of polyethylene terephthalate The mean particle diameter of 3.5 micrometers, The apatite support nonwoven fabric (magnitude 5mmx5mm) which made the porosity hydroxyapatite granulation of the calcium/P ratio 1.67 support 24% of the weight by heat-treatment is immersed into an avidin 5microg/ml water solution, and it is avidin 10 mg/m<sup>2</sup>. The adsorbed nonwoven fabric is created. this -- biotin-ized anti-Rota (Rota) IgG of a rabbit -- the amount was combined enough and the inspection sheet for rotavirus detection was created. the obtained inspection sheet -- using -- Sandwiches EIA -- the rotavirus was detected using law. That is, the rotavirus antigen in a rotavirus solution is caught with the above-mentioned detection sheet. After combining the anti-rotavirus alkaline phosphatase labelled antibody of a guinea pig with the rotavirus antigen on a detection sheet after that, it measured by coloring of the substrate DNP for EIA. Measurement wavelength was 405nm. When the dilution of a rotavirus antigen was changed and the sensibility of a detection sheet was examined, it has sensed to the amount of 0.062microg [/ml ] viruses. It is shown in drawing 1 as a related Fig. of the virus concentration and the absorbance by the above-mentioned measurement.

[0021] At about 1 appearance to a nonwoven fabric with a thickness of 0.2mm which consists of 50 % of the weight of example of comparison 1 polyethylene, and 50 % of the weight of polyethylene terephthalate The mean particle diameter of 3.5 micrometers, The porosity hydroxyapatite granulation of the calcium/P ratio 1.67 to the apatite support nonwoven fabric (magnitude 5mmx5mm) made to support 24% of the weight by heat-treatment After [ the anti-Rota (Rota) IgG antibody of a rabbit ] making an amount adsorb enough, Immersion processing was performed in the glutaraldehyde 0.05% solution, in order to mask the anti-Rota antibody non-adsorption site of the above-mentioned fiber aggregate, it was immersed in 4 times many block ace [ as this ] dilution, and the rotavirus detection sheet was created. Although detection of rotavirus 0.25microg/ml was tried on the same detection conditions as an example 1 using the obtained rotavirus detection sheet, an absorbance is 0.01 and was not able to be detected. On the other hand, when detection of rotavirus 0.25microg/ml was performed using the detection sheet created by the example 1, an absorbance is 0.95 and was fully detectable.

[0022]

[Effect of the Invention] If the detection sheet of this invention is used, there is no limit in the class of the antigen which should be fixed, or antibody, and there is also no problem of a stacking tendency, by easy actuation, the antigen or antibody in a biological liquid can be quickly detected by high sensitivity, and the detection kit [ that it is cheap and high sensitivity ] which can perform detection of an antigen or an antibody easily also in the small-scale hospital in which experts, such as a laboratory technician, are not present can be offered.

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**TECHNICAL FIELD**

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[Field of the Invention] This invention relates to the detection sheet, detection kit, and the detection approach of detecting the antigen or antibody in biological liquids, such as saliva, blood, lymph, and feces and urine.

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**PRIOR ART**

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[Description of the Prior Art] Although various clinical laboratory tests using an antigen-antibody reaction are conducted in recent years, a special facility and a special clinical laboratory technologist are required. Therefore, there is no special facility, it can inspect by easy actuation easily also in the small hospital in which a clinical laboratory technologist is not present, and the high sensitivity inspection sheet which can be made into an aid of a sick diagnosis is called for. Since it has the adsorption capacity in which calcium phosphate system compounds, such as hydroxyapatite, were excellent to protein, a nucleic acid, etc., this invention persons made the antigen or the antibody stick to the calcium phosphate system compound particle which paper and a nonwoven fabric were made to support, and the detection sheet on which an antigen-antibody reaction is made to perform has already been proposed (refer to Japanese-Patent-Application-No. No. 214706 [ six to ] specification).

However, it turned out that a problem is in that a calcium phosphate system compound has inadequate adsorption capacity, its adsorption site may not necessarily be fixed on the occasion of adsorption of an antibody to acidic protein, for example, a Fab fragment part may be adsorbed, and a stacking tendency. Therefore, in addition, the room of an improvement was left behind to the fixing method of the antigen which is hard to adsorb, or an antibody, or the effective joint approach of a calcium phosphate system compound and an antibody with the calcium phosphate system compound itself.

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**EFFECT OF THE INVENTION**

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[Effect of the Invention] If the detection sheet of this invention is used, there is no limit in the class of the antigen which should be fixed, or antibody, and there is also no problem of a stacking tendency, by easy actuation, the antigen or antibody in a biological liquid can be quickly detected by high sensitivity, and the detection kit [ that it is cheap and high sensitivity ] which can perform detection of an antigen or an antibody easily also in the small-scale hospital in which experts, such as a laboratory technician, are not present can be offered.

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**TECHNICAL PROBLEM**

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[Problem(s) to be Solved by the Invention] This invention cancels the trouble of the above-mentioned conventional technique, and aims at offering the detection sheet, detection kit, and the detection approach high sensitivity can detect the antigen or antibody in a biological liquid by easy actuation.

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## MEANS

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[Means for Solving the Problem] This invention is completed based on knowledge that the above-mentioned purpose can be attained by carrying out adsorption immobilization of avidin or the streptoavidin to the calcium phosphate system compound supported to the fiber aggregate. That is, the antigen by this invention or the detection sheet of an antibody is characterized by fixing avidin, streptoavidin, or these derivatives to the fiber aggregate with which the calcium/P ratio supported [ mean particle diameter ] the calcium phosphate system compound particle of 1.0–2.0 with 0.01 micrometers – 200 micrometers.

[0005] The detection sheet of this invention can also be offered where the antigen or antibody biotin-ized is combined with the avidin fixed as mentioned above, streptoavidin, or these derivatives. Moreover, by contacting the antigen by this invention, or the detection sheet of an antibody to the sample solution, the detection approach of the antigen by this invention or an antibody combines the antibody or antigen in a sample, is contacted in the solution of the labeled compound specifically combined with this antibody or an antigen after that, and is characterized by detecting the antigen-antibody complex by which the indicator was carried out.

[0006]

[Embodiment of the Invention] Avidin, streptoavidin, or these derivatives are made to stick to a calcium phosphate system compound in this invention. Here, as a derivative, the thing (NeutrAvidin), for example, new trad avidin, excluding the sugar chain part from avidin, ultra avidin, etc. are mentioned.

[0007] In this invention, a calcium phosphate system compound particle is used as avidin, streptoavidin, or an adsorption fixative of these derivatives. Here as a calcium phosphate system compound If calcium/P ratios are 1.0–2.0, various kinds of calcium phosphate system compounds can be used. For example, calcium<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub> and calcium<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>F<sub>2</sub>, calcium<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>Cl<sub>2</sub>, calcium<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>, calcium two P<sub>2</sub>O<sub>7</sub>, and calcium 4O(PO<sub>4</sub>)<sub>2</sub> And CaHPO<sub>4</sub> One sort chosen from inside or two sorts or more can be used. Hydroxyapatite and tricalcium phosphate are [ among these ] desirable, and what uses especially hydroxyapatite as a principal component is the most desirable. When using a fluorine apatite, it is desirable that the fluorine content in a total phosphorus acid calcium system compound is 5 or less % of the weight. The elution of a fluorine happens and is not desirable if fluorine content exceeds 5 % of the weight. These calcium phosphate system compounds are compoundable with a well-known wet synthesis method, a dry type synthesis method, etc.

[0008] Although it can prepare by corning the particle of a calcium phosphate system compound by carrying out spray drying of the slurry of for example, a calcium phosphate system compound, and calcinating this, not only this approach but the thing to prepare by other corning methods is possible. In addition, it is more desirable to select and use for the predetermined range [ for the purpose of the grain size of a particle ] with means, such as sieving. As for the calcium phosphate system compound particle to be used, it is desirable that mean particle diameter is 0.01–200 micrometers. It becomes it easy to condense a particle that mean particle diameter is less than 0.01 micrometers, and is not supported by homogeneity. Moreover, if it exceeds 200 micrometers, it will be hard coming to support to a nonwoven fabric, and the rate of support will fall remarkably.



[0009] Furthermore, calcium phosphate system compound particles are more than specific surface area of  $10\text{m}^2 / \text{g}$ , and, as for a calcium phosphate system compound particle, it is desirable that it is the porosity particle in which the primary particle with a pole diameter of 500–1000Å carried out condensation association. Adsorption capacity with a specific surface area sufficient by under  $10\text{m}^2 / \text{g}$  cannot be desired. Moreover, in order to adsorb and for protein etc. to enter into pore, it is desirable to have pore with a pole diameter of about 500–1000Å. Such a porosity particle can be manufactured by the well-known approach of arbitration.

[0010] In this invention, the fiber aggregate is used as support of the above calcium phosphate system compound particles. Here, paper or a nonwoven fabric is mentioned as the fiber aggregate which can be used. There is a method of adding this with an inner attachment method or a coating method as an approach of making paper supporting a calcium phosphate system compound particle, using a calcium phosphate system compound particle as a loading material, and manufacturing paper. In the case of an inner attachment method, after adding a calcium phosphate system compound particle and other additives and mixing enough, it can manufacture using the usual paper machine. Moreover, what is necessary is just to apply in the Hara paper combining a binder, in adopting a coating method. As a binder, there is especially no limit, for example, it can use sodium polyacrylate, polyvinyl alcohol, a latex, polyacrylic acid, polyethylene oxide, a carboxymethyl cellulose, polyester, etc.

[0011] Although the same approach as the case of paper can be adopted also when making a nonwoven fabric support a calcium phosphate system compound particle Even if there are few nonwoven fabrics with which at least 1 part of raw material fiber consists of thermoplastic macromolecule fiber, a calcium phosphate system compound particle is made to support with dry process or a wet method on a front face. Furthermore, subsequently It is desirable to adopt the approach of the thermoplastic macromolecule fiber in a nonwoven fabric of softening a front face at least and making this fiber front face fixing a calcium phosphate system compound particle, by heat treatment.

[0012] As for the amount of support of the calcium phosphate system compound to the fiber aggregate, it is usually preferably desirable to consider as 5 – 50 % of the weight one to 65% of the weight. At less than 1 % of the weight, since the amount of avidin, streptoavidin, or these derivatives used will become high if it is hard coming to adsorb avidin, streptoavidin, or these derivatives and exceeds 65 % of the weight, the amount of support of a calcium phosphate system compound is not economical.

[0013] In this way, it has a high absorption to the avidin of the manufactured fiber aggregate whose calcium phosphate system compound it has a calcium phosphate system compound layer as mentioned above in a front face, and is a proteinic kind at least, streptoavidin, and these derivatives, and adsorption immobilization of these can be carried out. In order to fix avidin, streptoavidin, or these derivatives to the fiber aggregate which supported the calcium phosphate system compound, it can carry out by the approach of immersion, brush coating, a spray coating cloth, etc. using the water solution of avidin, streptoavidin, or these derivatives. Moreover, avidin, streptoavidin, or the amount of immobilization of these derivatives is changed with the class and amount of a calcium phosphate system compound which are supported by the fiber aggregate, for example, is hydroxyapatite  $3.0\text{ g}/\text{m}^2$ . It sets to a support nonwoven fabric and is 5 – 20  $\text{mg}/\text{m}^2$ . It is desirable and is 10  $\text{mg}/\text{m}^2$ . Order is more desirable. 5  $\text{mg}/\text{m}^2$  There are many non-adsorption sites of hydroxyapatite at the following, and it is 20  $\text{mg}/\text{m}^2$ . If it exceeds, avidin, streptoavidin, or these amounts of derivatives are uneconomical.

[0014] Avidin, streptoavidin, and these derivatives Since it has very high compatibility to a biotin and another side and a biotin can be easily combined with the enzyme of an antibody or many By making the avidin fixed as mentioned above, streptoavidin or these derivatives, and the antigen or antibody biotin-ized react a fiber aggregate top -- a right stacking tendency -- with, after obtaining the inspection sheet which has the fixed antigen or antibody and contacting this to the sample solution, an antibody or an antigen is easily detectable by high sensitivity by making a labeled compound solution contact.

[0015] In this invention, although the antigen or antibody biotin-ized if needed, and the front stirrup made to react can also mask the non-adsorption site of the calcium phosphate system

compound particle supported to the fiber aggregate by the blocking agent after making it react, this process is not necessarily required. Moreover, the film for reinforcement or a sheet can also be made to adhere to the rear face of the nonwoven fabric of a detection sheet for improvement in the handling nature of the detection sheet of this invention. The film for reinforcement or a sheet may consist of paper or plastics.

[0016] Therefore, the detection kit of the antigen or antibody which consists of the above antigens, or the detection sheet and labeled compound solution of an antibody can be offered by this invention. Although the enzyme labelled antibody specifically combined with the antigen in a sample or an antibody as a labeled compound or an antigen, an isotope labelled antibody, or an antigen can be used, since detection actuation can be performed easily, enzyme labeling is desirable, without needing a special facility. It is detectable by easy approaches, such as visual observation or spectrometry, using the substrate which carries out coloration in the light or the ultraviolet region of the enzyme using an enzyme labelled antibody or an antigen.

[0017] As the enzyme used as a labeled compound, and a substrate, Substrates BCIP, NBT, or DNP are used to enzyme alkaline phosphatase, Substrates OPD and DAB or 4CN(s) are used to enzyme horseradish peroxidase, and Substrate pNPG, X-gal, or Bluo-gal is used to the beta-galactosidase, for example.

[0018] Although the detection sheet and the inspection approach by this invention mainly detect the existence or nonexistence of an antigen or an antibody, a certain amount of quantum of an antigen or an antibody is also possible for them by the comparison of the concentration of coloration, or measurement of an absorbance.

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**EXAMPLE**

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[Example] Next, this invention is not restricted by this although this invention is further explained in full detail based on an example.

[0020] At about 1 appearance to a nonwoven fabric with a thickness of 0.2mm which consists of 50 % of the weight of example 1 polyethylene, and 50 % of the weight of polyethylene terephthalate. The mean particle diameter of 3.5 micrometers, The apatite support nonwoven fabric (magnitude 5mmx5mm) which made the porosity hydroxyapatite granulation of the calcium/P ratio 1.67 support 24% of the weight by heat-treatment is immersed into an avidin 5microg/ml water solution, and it is avidin 10 mg/m<sup>2</sup>. The adsorbed nonwoven fabric is created. this -- biotin-ized anti-Rota (Rota) IgG of a rabbit -- the amount was combined enough and the inspection sheet for rotavirus detection was created. the obtained inspection sheet -- using -- Sandwiches EIA -- the rotavirus was detected using law. That is, the rotavirus antigen in a rotavirus solution is caught with the above-mentioned detection sheet. After combining the anti-rotavirus alkaline phosphatase labelled antibody of a guinea pig with the rotavirus antigen on a detection sheet after that, it measured by coloring of the substrate DNP for EIA. Measurement wavelength was 405nm. When the dilution of a rotavirus antigen was changed and the sensibility of a detection sheet was examined, it has sensed to the amount of 0.062microg [ /ml ] viruses. It is shown in drawing 1 as a related Fig. of the virus concentration and the absorbance by the above-mentioned measurement.

[0021] At about 1 appearance to a nonwoven fabric with a thickness of 0.2mm which consists of 50 % of the weight of example of comparison 1 polyethylene, and 50 % of the weight of polyethylene terephthalate. The mean particle diameter of 3.5 micrometers, The porosity hydroxyapatite granulation of the calcium/P ratio 1.67 to the apatite support nonwoven fabric (magnitude 5mmx5mm) made to support 24% of the weight by heat-treatment. After [ the anti-Rota (Rota) IgG antibody of a rabbit ] making an amount adsorb enough, Immersion processing was performed in the glutaraldehyde 0.05% solution, in order to mask the anti-Rota antibody non-adsorption site of the above-mentioned fiber aggregate, it was immersed in 4 times many block ace [ as this ] dilution, and the rotavirus detection sheet was created. Although detection of rotavirus 0.25microg/ml was tried on the same detection conditions as an example 1 using the obtained rotavirus detection sheet, an absorbance is 0.01 and was not able to be detected. On the other hand, when detection of rotavirus 0.25microg/ml was performed using the detection sheet created by the example 1, an absorbance is 0.95 and was fully detectable.

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[Translation done.]

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3.In the drawings, any words are not translated.

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**DESCRIPTION OF DRAWINGS**

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[Brief Description of the Drawings]

[Drawing 1] It is the graphical representation showing the detection result of the rotavirus in an example 1.

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[Translation done.]



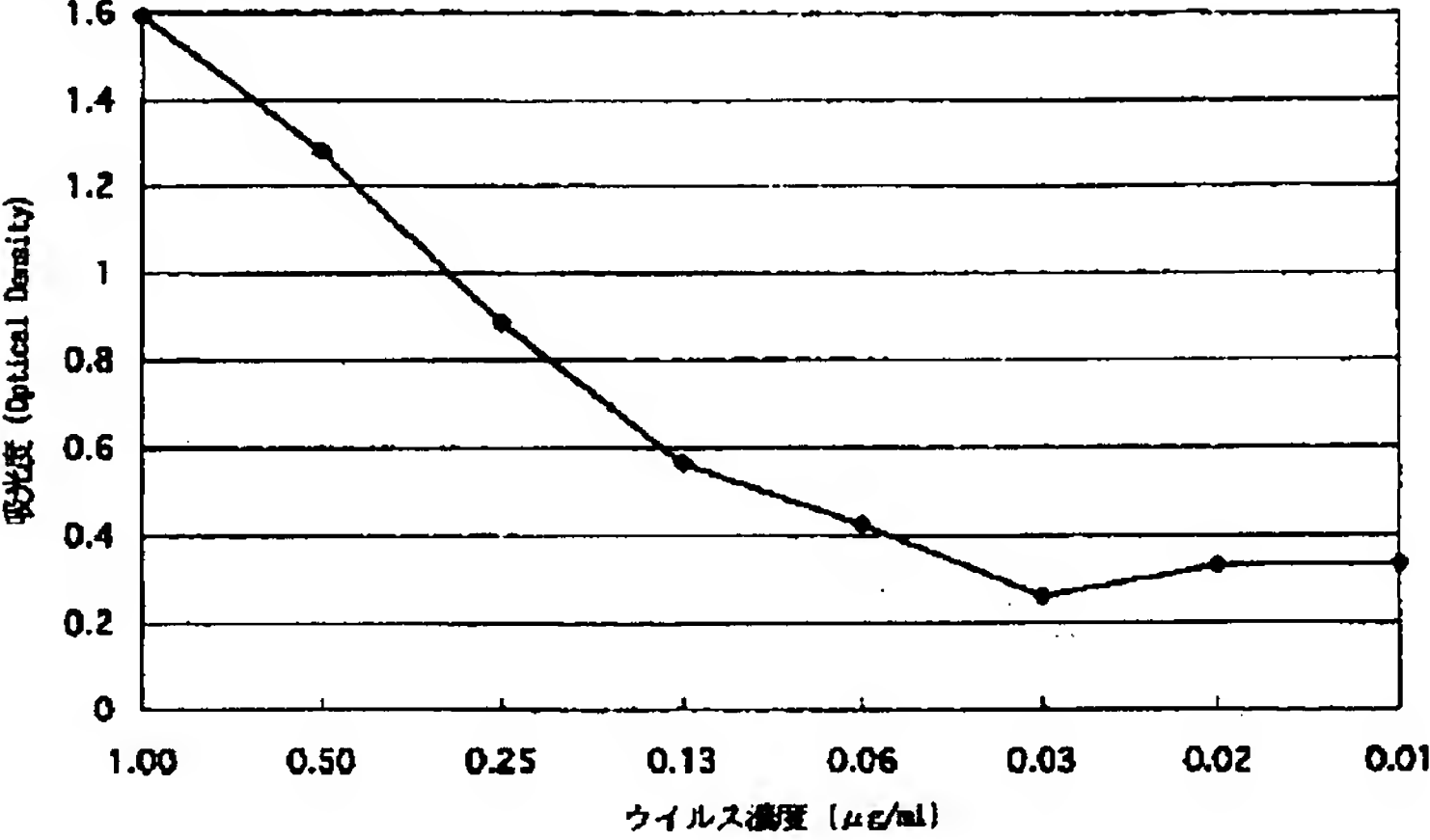
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DRAWINGS

[Drawing 1]



[Translation done.]